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THE EFFECT OF CYTOCHALASIN B UPON TAIL RESORPTION AND METAMORPHOSIS IN TEN SPECIES OF ASCIDIANS[1]

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Metamorphosis of the free swimming tadpole larva is a cardinal event in the life cycle of all ascidians, with the exception of a few anurous species in the families Molgulidae and Styelidae, which exhibit direct development (Berrill, 1931; Millar, 1971). There are many variations in the details of metamorphosis in different families. The following events are characteristic of most species.

Metamorphosis begins at the moment the larva settles. Settling is effected by the secretion of a sticky substance by the adhesive papillae and may involve rapid elongation or eversion of the papillae in some species. Settling is followed by resorption of the tail, retraction of the adhesive papillae, retraction or collapse of the sensory vesicle, emigration (in some species) of blood cells across the epidermis into the tunic, enlargement or elongation of epidermal ampullae, expansion of the tunic of the trunk, loss of the outer cuticular layer of tunic (comprising the fins of the tail), and a gradual rotation of the viscera and siphons through an arc of about 90 degrees. The axial complex of the tail is phagocytized and parts of the larval neurosensory system later undergo histolysis.

Postlarval development is highly variable. In some species the visceral organs are well differentiated in the larva and feeding begins within one to several hours (Berrill, 1935; Cloney, 1972). In other species, rudiments of the viscera are less well developed and must undergo differentiation over a period of several days before feeding begins (Grave, 1926, 1944; Cloney, 1961).

The morphogenetic movements associated with tail resorption have been studied extensively. In representative species of the families Polyclinidae, Clavelinidae, and Didemnidae (classification, Berrill, 1950), the caudal epidermis contracts during tail resorption and forces the axial complex (notochord, muscle and nerve cord) into the trunk (Cloney, 1963, 1972; Cloney and Lash, 1972). Contraction of the epidermis in *Amaroucium constellatum, Distaplia occidentalis* and *Diplosoma macdonaldi* is associated with the alignment of prominent arrays of cytoplasmic filaments in the epidermal cells (Cloney, 1966, 1972). In *Boltenia villosa* (family pyuridae) the notochordal cells appear to be contractile and the epidermis is evidently passive; changes in the organization of cytoplasmic filaments were found only in the notochordal cells (Cloney, 1969).

In the past few years, oriented arrays of cytoplasmic filaments (microfilaments) have been found in a wide variety of metazoan cells, which had been fixed while

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undergoing movements or changes of shape (*e.g.,* Baker and Schroeder, 1967; Schroeder, 1968, 1970; Arnold, 1969; Tilney and Marsland, 1969; Szollosi, 1970; Wessells, Spooner, Ash, Bradley, Luduena, Taylor, Wrenn, and Yamada, 1971; Crawford, Cloney and Cahn, 1972). The discovery that cytochalasin B (CCB) interferes with cytokinesis (Carter, 1967), and a variety of morphogenetic movements, and simultaneously interferes with the organization of microfilaments (Schroeder, 1969, 1970, 1972; Wessells *et al.,* 1971; Wrenn, 1971; Yamada, Spooner and Wessells, 1971; Spooner and Wessells, 1972) suggested a convenient method of analyzing morphogenetic movements in a variety of ascidians.

We have determined that CCB reversibly inhibits tail resorption and disrupts the organization of arrays of cytoplasmic filaments in the contractile epithelial cells (Lash, Cloney and Minor, 1970; Cloney, Lash and Minor, 1971; Cloney, 1972). In this paper we will describe the effect of CCB on tail resorption in ten species of ascidians including representatives of 8 different families and 3 suborders. We will show that there is a considerable variation in the effective concentration of the drug when it is used with different groups of ascidians, and that within a single species, discrete morphogenetic events, characteristic of metamorphosis, are differentially affected.

Materials and Methods

Ten species of ascidians, representing the following suborders (Berrill, 1950), were used for the cytochalasin B experiments: Aplousobranchia; *Distaplia occidentalis, Diplosoma macdonaldi, Amaroucium constellatum.* Phlebobranchia; *Ciona intestinalis, Perophora viridis.* Stolidobranchia; *Botryllus schlosseri, Styela partita, Boltenia villosa, Molgula citrina, M. manhattensis.* Ultrastructural studies of the localization of cytoplasmic filaments were performed on *D. occidentalis, D. macdonaldi, A. constellatum, B. schlosseri, B. villosa, M. manhattensis,* the phlebobranch *Ascidia callosa,* and the stolidobranchs *Pyura haustor* and *Styela gibbsii* (methods of fixation have been described elsewhere; Cloney, 1964, 1966, 1972).

D. occidentalis, D. macdonaldi, B. villosa, P. haustor, S. gibbsii and *A. callosa* were obtained near the Friday Harbor Laboratories, University of Washington. All other species were obtained at the Marine Biological Laboratory, Woods Hole, Massachusetts. Tadpoles of oviparous species (*C. intestinalis, B. villosa, M. manhattensis, S. partita*) were used for experimentation 8–18 hours after hatching. Since the tadpoles from the ovoviviparous species (*D. occidentalis, D. macdonaldi, A. constellatum, P. viridis, B. schlosseri, M. citrina*) metamorphose shortly after they escape from the adult colonies, these species were treated with cytochalasin B within minutes or a few hours after the tadpoles were released.

For experimental observation, the tadpoles were placed in culture dishes containing filtered sea water (FSW), or FSW plus cytochalasin B. Periodic observations were made with either a dissecting microscope, a bright field or a Zeiss Nomarski differential interference microscope. Time lapse films of some species (*D. occidentalis, D. macdonaldi, B. villosa, A. constellatum, C. intestinalis, P. viridis, M. citrina, M. manhattensis*) were made with a Series 500 Sage cinephotomicrographic appartus, or a Kodak Reflex S, equipped with an L. W. photo intervalometer. Exposure intervals ranged from 0.5 to 8 frames per second.

Cytochalasin B was generously provided by S. B. Carter of Imperial Chemical

TABLE I

Effective concentration of cytochalasin B which arrests or prevents the onset of tail resorption (micrograms/ml of sea water)

	CCB dosage	Specimens observed
Order Enterogona		
Suborder Aplousobranchiata		
Distaplia occidentalis	0.25–0.5	800
Diplosoma macdonaldi	0.25–0.5	600
Amaroucium constellatum	0.5 –1.0	3000
Suborder Phlebobranchiata		
Ciona intestinalis	1.0 –2.0	2000
Perophera viridis	1.0 –2.0	70
Order Pleurogona		
Suborder Stolidobranchiata		
Botryllus schlosseri	5–7	1000
Boltenia villosa	5–10	200
Stylela partita	5–10	1000
Molgula citrina	10–15	100
Molgula manhattensis	15–20	2000

Industries Ltd., Macclesfield, Cheshire, England. A stock solution was prepared by dissolving 10 mg of CCB in 10 ml of dimethylsulfoxide (DMSO). Aliquots of 0.5 ml in glass tubes were kept at −10° C until use. A fresh stock solution was made every 10–14 days, since cytochalasin B absorbs to the glass container, and the effective dose level (by volume) increases with time. For experimental use, the stock solution was diluted with fresh FSW to concentrations ranging from 0.25 μg/ml to 50 μg/ml. Controls were treated with DMSO in FSW at the same concentration used in the cytochalasin B experiments.

RESULTS

Inhibition of metamorphosis

The intiation of metamorphosis can be completely inhibited by cytochalasin B, in all ten species of ascidians tested (Table I). It is clear that the effective concentration of the drug is distinctly different in the three suborders represented.

Arresting tail resorption

All ten species were tested to determine the effective concentration that would arrest tail resorption after the process had begun. The effective concentration was identical to that which prevented the initiation of tail resorption (Table I).

Reversal of the effects

Aplousobranchs. If the concentrations are minimal, and if the tadpoles are washed shortly after tail resorption is stopped, the effects of cytochalasin B are reversible. When CCB is added during tail resorption, the tail tends to push out again while in the presence of the drug. This is due to the relaxation of the

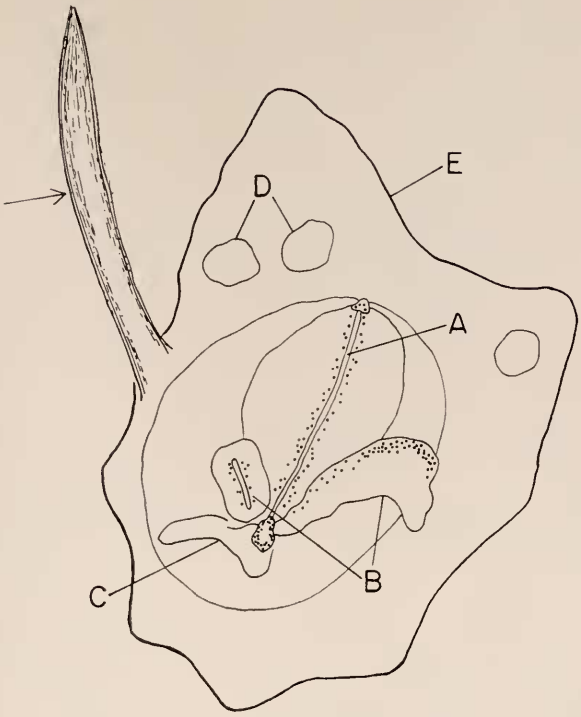

FIGURE 1. *Botryllus schlosseri.* Tadpole was kept in cytochalasin B (10 μg/ml) until signs of metamorphosis were observed (discharge of adhesive papillae, tunic swelling), then rinsed in fresh sea water. After seven days, the unresorbed tail (arrow) still protruded from the developing organism. Scale line is 1 mm. Abbreviations are: A, Endostyle of branchial basket; B, Degenerating (right) and take-over buds; C, Intestine; D, Ampullae; E, Tunic.

epithelial cells and uncoiling of the axial complex. When treated larvae are placed in fresh sea water, the tails begin to shorten again within 0.5 to 4 minutes, depending upon the duration of exposure to CCB (*A. constellatum, B. schlosseri, C. intestinalis, P. viridis*). In most instances complete recovery occurs only if the animals are washed within a few minutes after the tail stops shortening (*D. occidentalis* and *D. macdonaldi*), but many of the larvae of *A. constellatum* can recover and completely resorb their tails after periods as long as 35 minutes in the presence of CCB. Variations in the length of time in which recovery can be effected have been observed, and this may reflect differences in batches of tadpoles.

Tail resorption in *A. constellatum* was reversed four consecutive times within 20 minutes (four additions of CCB, four rinses). After the fourth reversal, the epidermis ruptured and the axial complex uncoiled. The epidermal cells separated from their neighbors, rounded up, and appeared as isolated beads on the surface of the axial complex.

Phlebobranchs. With the exception of a longer recovery time in the phlebo-

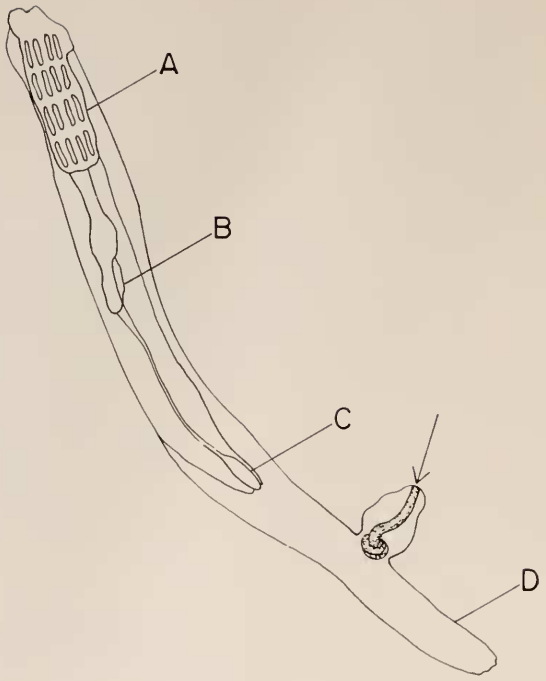

FIGURE 2. *Amaroucium constellatum*. Tadpole was treated with cytochalasin B (5 μg/ml), and rinsed before tail resorption was initiated. The tail underwent partial resorption (arrow). Scale line is 1 mm. Abbreviations are: A, Branchial basket; B, Intestine; C, Heart; D, Tunic.

branchs, the re-extension of the tail in the presence of CCB was similar to the events recorded in the aplousobranchs. Re-extension of the tail and recovery of movement sometimes took as long as 2–3 hours. Repetitive reversal was not tried with these species.

Stolidobranchs. In all five species examined, the stolidobranchs showed the following attributes: (1) They required the highest concentration of CCB to effectively inhibit tail resorption, (2) they required the longest time for CCB to stop tail resorption, and (3) they required the longest time for re-extension to occur. After adding CCB it took up to ten minutes for tail resorption to cease, and in some instances (*S. partita*) reinitiation of tail resorption after washing took as long as 4–5 hours. The completion of tail resorption sometimes took as long as 10 hours (*S. partita, M. manhattensis*).

Cytochalasin B, tail resorption, and metamorphosis

Reversal of the effects of cytochalasin B does not always result in normal tail resorption, but unless the concentration is high (2–3 times the minimum dose), general metamorphosis and postlarval development resumes. Frequently the

organisms can continue development with an unresorbed, or partly resorbed tail protruding from the body wall (Fig. 1). These animals developed and behaved normally during the time of observation (8–10 days).

If tadpoles (*A. constellatum, B. schlosseri*) are placed in CCB, then rinsed before tail resorption is initiated, the process of tail resorption and metamorphosis is usually normal. In a few cases the tail did not undergo completely normal resorption. In *A. constellatum,* after completion of tail resorption, a compact, cone-shaped mass of contracted caudal epidermal cells persists near the base of the former tail (Fig. 2). These epidermal cells were often unable to contract further and invert the epithelial cone as occurs normally (Cloney, 1966). In *B. schlosseri,* if resorption is not completed, a small stump of tissue remains covered by a thin epithelium. In neither instance do these minor abnormalities have any effect upon further postlarval development.

Observations on normally metamorphosing tadpoles of *A. constellatum* have shown that tadpoles occasionally undergo metamorphosis without tail resorption. Excision of the tail of *A. constellatum, D. occidentalis, D. macdonaldi, B. villosa, P. viridis,* and *B. schlosseri* does not interfere with subsequent metamorphosis of the trunk. Thus inhibition of tail resorption with cytochalasin B would not necessarily be expected to affect the other characteristic events of metamorphosis.

Cytochalasin B and metamorphosis

In the aplousobranchs (*D. occidentalis, D. macdonaldi,* and *A. constellatum*), both the evanescent larval organs and the visceral organs of significance in postlarval life are well differentiated. Although *M. citrina* is ovoviviparous, and undergoes metamorphosis shortly after hatching, its structure is relatively simple (Grave, 1926). The other species tested in the stolidobranchs (*M. manhattensis, B. villosa, S. partita*), as well as *C. intestinalis,* are oviparous species. After external fertilization, and a relatively short (8–32 hours, 12°–22° C) period of development, a simple tadpole is hatched and may swim for several days before undergoing metamorphosis. *M. manhattensis, M. citrina, B. villosa, S. partita,* and *C. intestinalis* all undergo extensive differentiation after metamorphosis, before the adult organs become functional.

With these two distinctive types of development, from highly differentiated to simple larvae, it was possible to test the effects of cytochalasin B on processes other than tail resorption. As mentioned previously, the trunk can undergo normal metamorphosis even if the tail is only partly resorbed (*A. constellatum, D. occidentalis, B. schlosseri, D. macdonaldi, B. villosa*). At minimal dosage levels and continuous treatment, *A. constellatum* and *B. schlosseri* can develop with the tail protruding from the body wall. After several days the tail undergoes histolysis and falls off.

Tadpoles of *A. constellatum* and *B. schlosseri* were submitted to two types of treatment. In one, the tadpoles were continually exposed to cytochalasin B for periods ranging up to 7 days. The other treatment consisted of exposing animals to different concentrations of the drug, then rinsing them with FSW. In concentrations of 1.0 μg/ml of CCB, specimens of *A. constellatum* fail to resorb their tails, but otherwise develop normally.

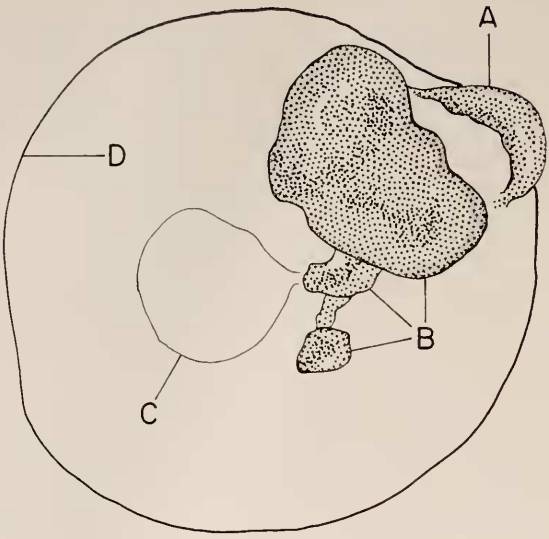

FIGURE 3. *Botryllus schlosseri.* Tadpole was kept continuously in cytochlasin B (10 μg/ml) for four days. The terata formed had a beating heart (A) and disorganized tissue (B). Scale line is 1 mm. Abbreviations are: A, Heart; B, Tissue mass; C, Vesicle; D, Tunic.

In control dishes containing only filtered sea water, up to five per cent of these tadpoles metamorphosed without resorbing their tails.

After a few hours, sometimes as long as 10 hours, in solutions of CCB, the caudal epidermal cells separate, and collect in clumps of cells along the length of the tail. The time at which this happens varies, and may be related to the developmental age of the tadpole. Tadpoles which metamorphose immediately after spawning may be older than those tadpoles which swim for longer periods before settling. All fully developed tadpoles are released each day in a brief period following exposure to light, whereas the development of the embryos is probably a continuous process. Thus with periodic release, the tadpoles would differ slightly in age.

In 5.0 μg/ml of CCB, the tail of *A. constellatum* is not resorbed, the tunic of the trunk however does begin to swell (a manifestation of metamorphosis), although there are few internal changes in the organism associated with metamorphosis, such as rotation of the visceral organs and opening of the siphons. Two-thirds of these animals show some inhibition of metamorphosis (30 tadpoles tested). The heart continues to beat for periods up to 4–5 days, but the animal remains small and malformed, and does not live past this time. At higher concentration, the animals die within a few days. Between 5–10 μg/ml of CCB there appears to be little, if any increase in size. It is probable that cytokinesis is strongly inhibited. At concentrations of 10 μg/ml, both tail resorption and general metamorphosis are completely inhibited (30 tadpoles tested).

With minor differences, the results of prolonged exposure of *B. schlosseri* to CCB are similar to those obtained with *A. constellatum*. The cytoplasmic filaments associated with cellular contraction are in the apical region of the epidermal cells of *A. constellatum* (Cloney, 1966), whereas they are in the basal part of the contracting epidermal cells of *B. schlosseri* (Minor and Lash, unpublished). At increasing concentrations (10 μg/ml), *B. schlosseri* acquired more abnormalities, and there is very little evidence of growth or morphogenesis. At the higher dosage levels, the organism transforms into a small (1.5 mm diameter) disorganized mass of tissue with a beating heart (Fig. 3). These terata continue "living" for a few days, then degenerate. This is further evidence that higher concentrations of cytochalasin B are required to inhibit growth and morphogenesis than is necessary to inhibit tail resorption.

Discussion

Configurational changes of a variety of cells have been correlated with the presence, in specific localized areas of the cytoplasm, of oriented arrays of 50–70 Å (diameter) filaments.

Organized arrays of filaments associated with the plasmalemma of the cleavage furrow have been implicated in the mechanism of cytokinesis in hydrozoans (Schroeder, 1968; Szollosi, 1970), polychaetes (Szollosi, 1970), cephalopods (Arnold, 1969), echinoids (Tilney and Marsland, 1969; Schroeder, 1969; 1972), and amphibians (Selman and Perry, 1970; Bluemink, 1970). They have also been implicated in the contraction of cells involved in the morphogenesis of the neural tube (Baker and Schroeder, 1967; Schroeder, 1970; Burnside, 1971), the salivary gland, pancreas, and oviduct (Spooner and Wessells, 1972; Wessells and Evans, 1968; Wrenn, 1971). In addition, these filaments appear to be involved in the movement of axonal growth cones (Yamada *et al.*, 1971), slime mold movements (Wohlforth-Bottermann, 1964), movements of glial cells (Spooner, Yamada and Wessells, 1971), platelet contraction (Shepro, Belamarich, Robblee and Chao, 1970), macrophage movement and endocytosis (Allison, Davies and de Detris, 1971), chemotaxis of polymorphonuclear leukocytes (Becker, Davis, Estensen and Quie, 1972), amoeboid movement (Nachmias, 1968), and the movement of human lymphocytes (Smith, Ridler and Faunch, 1967). It has even been proposed by Jones (1966) that cell reaggregation (specifically, sponge cells) rely upon a mechanism involving cytoplasmic contractile elements (*viz.* cytoplasmic filaments).

For all species of ascidians thus far analyzed (*D. occidentalis, D. macdonaldi, A. constellatum, B. villosa, B. schlosseri*), the evidence strongly suggests that the cytoplasmic filaments provide, in an as yet unknown manner, the motive force necessary for tail resorption (Cloney, 1966; 1969; 1972). Projecting from data obtained from the variety of systems previously mentioned, it was predicted that cytochalasin B would interfere with tail resorption. In all ten species tested, representing three different suborders and eight different families, cytochalasin B prevented the initiation of tail resorption. If treated with the drug after tail resorption had begun, shortening of the tail was arrested. In the aplousobranchs, phlebobranchs, and the stolidobranchs (with the exception of *B. villosa*), the contractile tissues then relaxed and the tail began to push out again. In *A. constellatum* this process of reversal could be repeated four times.

Ultrastructural studies of *A. constellatum* and *D. occidentalis* have shown that when specimens are fixed immediately after tail resorption is inhibited with CCB, most of the central and subterminal arrays of filaments which normally span the apex of the contracting epidermal cells are disorganized. The only remaining organized arrays of filaments are short tufts or patches attached to the plasmalemma (Lash, Cloney and Minor, 1970; Cloney, 1972). The presence of the terminal tufts of filaments suggests that treatment with CCB does not completely degrade the filaments into subunits. It is more likely that the drug disrupts the binding force between overlapping unattached filaments.

Although it has been reported that cytochalasin B has a pronounced inhibitory effect upon polysaccharide synthesis (Sanger and Holzter, 1972), data from others indicate that this observed inhibition is an artifact, and that it is the result of cytochalasin B rapidly inhibiting the transport of such molecules as glucose, glucosamine, D-2-deoxyglucose (Estensen and Plagemann, 1972; Kletzien, Perdue and Springer, 1972; Zigmond and Hirsch, 1972) and nucleosides (Plagemann and Estensen, 1972). The effect of cytochalasin B on ascidians reported in this paper occurs so rapidly that one would not expect general synthetic processes to be involved in the mechanism of inhibition. It is not however, possible to determine whether CCB acts directly or indirectly upon the filaments.

The effective range of concentration of cytochalasin B which prevents tail resorption exhibited a striking phylogenetic correlation. Tail resorption in all three species of aplousobranchs (*D. occidentalis, D. macdonaldi, A. constellatum*) was effectively blocked at a concentration range of 0.25–1 μg/ml. In the phlebobranchs (*C. intestinalis* and *P. viridis*) the effective concentration range was 1–2 μg/ml. In the stolidobranchs (*B. schlosseri, B. villosa, S. partita, M. citrina* and *M. manhattensis*), resorption was blocked in a concentration range from 5–20 μg/ml. The two molgulids tested (representing the most phylogenetically advanced family of this suborder, Berrill (1950)) required the highest concentration (*M. citrina*, 10–15 μg/ml; *M. manhattensis*, 15–20 μg/ml). It is not possible to state whether the subordinal relationship to effective concentration will hold true when more species are tested.

There are several possible explanations for the differences in effective concentrations found. In the aplousobranchs, which are affected by the *lowest* concentrations, the most superficial tissue, the caudal epidermis, is contractile and the organized arrays of filaments are localized in the most superficial region (apical cytoplasm) of these cells (Cloney, 1966). In contrast, in the stolidobranchs, which require a 5–10 fold higher concentration, the organized arrays of filaments are located in the basal region of the epidermal cells (*B. schlosseri*), or in the notochordal cells (*B. villosa*). Thus there is a correlation between the effective CCB concentration and the locus of the contractile organelles. The localization of cytoplasmic filaments in 13 species of ascidians is summarized in Table II. In *D. occidentalis, D. macdonaldi, A. constellatum, C. intestinalis, A. callosa, B. schlosseri, B. villosa, M. manhattensis, S. gibbsii* and *P. haustor* the localization of filaments (as indicated by the symbol +) was determined by electron microscopy (Cloney, 1966, 1969, 1972; Lash, Cloney and Minor, 1970; Wessells, Spooner, Ash, Bradley, Ludena, Taylor, Wrenn and Yamada, 1971; Cloney, unpublished; Minor and Lash, unpublished). The localization of filaments in *P. viridis* was deduced by

TABLE II

Localization of major arrays of cytoplasmic filaments in thirteen species of ascidians undergoing tail resorption

	Caudal epidermis (apical)	Caudal epidermis (basal)	Notochord
Order Enterogona			
Suborder Aplousobranchiata			
Distaplia occidentalis	+		
Diplosoma macdonaldi	+		
Amaroucium constellatum	+		
Suborder Phlebobranchiata			
Ciona intestinalis	+		
Perophora viridis	+		
Ascidia callosa	+		
Order Pleurogona			
Suborder Stolidobranchiata			
Botryllus schlosseri		+	
Boltenia villosa			+
Molguia citrina			P
Molgula manhattensis			+
Pyura haustor			+
Styela gibbsii			+
Styela partita			P

analysis for birefringence (Lash and Reigart, 1965). For the other two species listed in the table, the probable position of the cytoplasmic filaments (as indicated with the symbol P) was deduced from time-lapse cinemicrographic observations, and changes in cell shape during tail resorption. Although the epidermis and notochord, in different species, appear to contain the major arrays of cytoplasmic filaments, the localization of filaments in these principal sites does not rule out the possibility of their occurrence in additional locations.

The correlation between effective CCB concentration and the locus of the contractile organelles suggests that the drug must be used in higher concentrations when the site of action is deeper within the organism. This would appear more reasonable if the data on *B. schlosseri* were not known. The filaments in the epidermal cells of *B. schlosseri* are only a few microns deeper within the cytoplasm of the epidermal cells than they are in epidermal cells of the aplousobranchs tested. Furthermore the contractile ampullae of *B. schlosseri* are composed of epidermal cells which have filaments in the basal cytoplasm (DeSanto and Dudley, 1969), and are 5–10 times more sensitive to the drug than the caudal epidermis.

An alternate explanation for the results is that the permeability of ascidian tissues to CCB varies along phylogenetic lines as well as among tissues of the same organism. Recent experiments by Bluemink and Luchtel (personal communication) on *Xenopus* eggs indicate that uncleaved eggs are impermeable to CCB. Localized inhibitory effects of the drug on cleavage are manifested when CCB is injected into egg cytoplasm.

Since nothing is yet known of the molecular aspects of the cytoplasmic filaments, or how their alignment is translated into motive force, it is not possible with assurance to say whether there may be different mechanistic principles involved

in the different tissues. Recent work has indicated that 50–70 Å cytoplasmic filaments in many different cell types are actin, or very similar to actin (Jones, 1966; Ishikawa, Bischoff and Holtzer, 1969; Jones and Kemp, 1970; Tilney and Mooseker, 1971; Pollard, 1972). The cytoplasmic filaments in the contractile epidermal cells of *Distaplia occidentalis* have also been shown to bind heavy meromyosin and form "decorated filaments" like those that have been described in other non-muscular cells (Cloney, unpublished).

It appears that wherever contractile processes are associated with cytoplasmic filaments, these processes can be inhibited or prevented by treatment with cytochalasin B. Whatever the multiple effects of CCB may be, its disorganizing effect on arrays of actin-like filaments in non-muscular cells seems to be wide spread.

Tail resorption is just one event in ascidian metamorphosis. With higher concentrations of CCB, other metamorphic events are affected. Dosage levels which will affect other specific cellular processes have not been worked out in detail, but in all instances the minimum concentration inhibiting tail resorption is compatible with subsequent development in the aplousobranchs and phlebobranchs. In the stolidobranchs the effective concentration is much higher (5–20 μg/ml), and these higher concentrations result in developmental impairments. In some extreme instances, terata are formed. These terata are composed of a disorganized tissue mass and a pulsating heart. In general, contraction of striated muscle was not inhibited, but the results were variable. *A. constellatum* tadpoles swim for days in cytochalasin B, whereas caudal muscular activity is inhibited in *D. occidentalis* after 6 hours (Cloney, 1972). Although evidence is mounting that cytochalasin B may have multiple effects upon cellular processes, one general process which is inhibited is a contraction associated with the presence of cytoplasmic filaments in non-muscular cells.

The authors are grateful to S. B. Carter for providing a supply of cytochalasin B.

Summary

Cytochalasin B reversibly inhibits tail resorption and disrupts the organization of arrays of cytoplasmic filaments in the contractile cells. The effects of cytochalasin B on tail resorption and metamorphosis has been examined in ten species of ascidians, including representatives of 8 different families and 3 suborders. There is a considerable variation in the effective concentration of the drug when it is used with different groups of ascidians, and within a single species, discrete morphogenetic events characteristic of metamorphosis are differentially affected. The effective range of concentration of cytochalasin B which prevents tail resorption exhibited a striking phylogenetic correlation.

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